VARIATIONS OF THE INDIVIDUAL BLOOD PLASMA AMINO ACID NITROGEN LEVEL

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Several papers (1–3) have been published recently on the subject of the amino nitrogen of human blood plasma as determined by Hamilton and Van Slyke's ninhydrin method (1).

The values reported for the blood plasma filtrates of persons in the post-absorptive state vary from 3.35 to 5.00 (1), 2.3 to 7.3 (2), and 3.37 to 4.97 (3) mg. of amino nitrogen per 100 ml. of plasma.

It has also been reported (3, 4) that significant differences of the amino nitrogen level are found when samples are taken from the same person on different days.

The above reports do not show whether each individual develops his own average amino nitrogen level or passes through the full range of observed values. Only a large number of analyses carried out on blood samples from a large number of persons can absolutely answer this question. However, the results of a limited number of analyses can be dealt with by means of statistical techniques.

We have, therefore, endeavored to follow the fluctuations of the amino nitrogen contents of human blood plasma by Van Slyke's ninhydrin technique for periods of more than 3 months.

The blood samples were taken during the summer and autumn months of 1944 from two distinctly different groups of people: (1) presumably healthy individuals (two men and three women) of ages between 21 and 38 years; (2) a group of twelve males, mental patients, physically well, of ages between 29 and 77 years, who were under strict dietary control for well over 1 year as participants in a nutritional project being conducted at Elgin. Differences due to environment or working conditions can, therefore, be minimized as factors affecting the data reported below.

Method

Samples were taken before breakfast and the oxalated plasma treated according to Hamilton and Van Slyke's ninhydrin method (1). No correction was made for plasma urea concentration, since no urea concentration of more than 18 mg. of urea nitrogen per 100 ml. of plasma had been found.
The average difference between duplicate determinations of plasma was 1.5 per cent or 0.064 mg. per cent of amino nitrogen. The analytical results were checked daily by including a sample of tyrosine or tyrosine and glutamic acid with the plasma filtrates. Because recoveries of amino nitrogen from these amino acids were consistently accurate to better than 1 per cent, two of the more experienced operators of the Van Slyke manometer apparatus did single determinations on many of the later samples (see Fig. 1).

The data reported in Fig. 1 were first used to determine the average of each individual (Table I, Column b). Then the deviations from this aver-
age and the optimum standard deviation estimate, \( s \), were computed, 
\[
\sigma = \sqrt{\frac{\sum (X - \bar{X})^2}{n-1}},
\]
where \( X \) is the analytical result, \( \bar{X} \) the arithmetical mean of all the data for one individual, and \( n \) the number of determinations. The confidence limits of the individual averages were then determined for the 95 per cent probability point (Table I, Column e)

\[
P_{95\%} = \pm \frac{ts}{\sqrt{n}},
\]
where \( t \) is the probability factor for the 95 per cent point, \( n - 1 \) the degrees of freedom (6, 5), and \( s \) the optimum standard deviation estimate (Table I, Column d). As an example the average of eighteen determinations carried out on subject C. T. is 4.65 mg. per cent with a 95 per cent confidence limit of \( \pm 0.07 \). There is, therefore, only 1 chance in 20 that the "true" average of this person's
amino nitrogen is outside the limits 4.72 and 4.58 mg. per cent. There is an even chance that any fasting sample taken from this subject will show

**Table I**

**Results of Statistical Computations**

The results in Columns b, d, and e are expressed in mg. per cent of amino acid nitrogen.

<table>
<thead>
<tr>
<th>Subject</th>
<th>No. of determinations, n</th>
<th>Average $X$</th>
<th>$\sum X^2$</th>
<th>Optimum s.d. estimate, $s$</th>
<th>Confidence limits (95 per cent probability) of average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td></td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>G. B.</td>
<td>11</td>
<td>4.36</td>
<td>2.410</td>
<td>0.490</td>
<td>0.31</td>
</tr>
<tr>
<td>J. C.</td>
<td>9</td>
<td>3.71</td>
<td>2.841</td>
<td>0.505</td>
<td>0.41</td>
</tr>
<tr>
<td>M. H.</td>
<td>9</td>
<td>3.94</td>
<td>1.327</td>
<td>0.406</td>
<td>0.28</td>
</tr>
<tr>
<td>J. N.</td>
<td>6</td>
<td>4.04</td>
<td>0.209</td>
<td>0.203</td>
<td>0.17</td>
</tr>
<tr>
<td>E. N.</td>
<td>7</td>
<td>3.98</td>
<td>1.031</td>
<td>0.415</td>
<td>0.33</td>
</tr>
</tbody>
</table>

| Psychotic |                         | (a)         | (b)         | (c)                         | (d)                                                  | (e)                                                  |
| S. T.     | 18                      | 4.08        | 0.725       | 0.201                        | 0.09                                                 |
| T. S.     | 18                      | 4.18        | 2.378       | 0.374                        | 0.18                                                 |
| E. R.     | 18                      | 3.96        | 0.472       | 0.166                        | 0.08                                                 |
| W. H.     | 18                      | 4.75        | 2.907       | 0.413                        | 0.20                                                 |
| D. S.     | 19                      | 4.17        | 1.830       | 0.310                        | 0.15                                                 |
| P. W.     | 18                      | 4.00        | 0.733       | 0.208                        | 0.10                                                 |
| C. T.     | 18                      | 4.65        | 1.381       | 0.149                        | 0.07                                                 |
| H. M.     | 18                      | 4.37        | 1.806       | 0.225                        | 0.16                                                 |
| H. B.     | 18                      | 4.45        | 1.100       | 0.254                        | 0.13                                                 |
| W. W.     | 20                      | 4.28        | 1.415       | 0.273                        | 0.14                                                 |
| H. J.     | 18                      | 4.73        | 1.111       | 0.255                        | 0.13                                                 |
| D. H.     | 19                      | 4.08        | 1.346       | 0.273                        | 0.13                                                 |
| 17 individuals |                       | 4.22        | 1.472       | 0.297*                       | 0.18†                                                 |

* Optimum standard deviation estimate = $\sqrt{\frac{\sum (\text{individual average} - 4.22)^2}{17 - 1}}$

† 95 per cent probability = $t_{16}/\sqrt{n} = \frac{2.1 \times 0.297}{\sqrt{17}}$; $t_{16}$ = the probability factor for 16° of freedom (5).

a deviation from 4.65 mg. per cent by more or less than 0.149 mg. per cent (Table I, Column d).

The average of all the 262 determinations was found to be 4.26 mg. per cent. If, however, the seventeen individual averages (Table I, Column b) are averaged, the more reliable total average of 4.22 mg. per cent is found.
The optimum standard deviation of the individual average from 4.22 mg. per cent is computed to be 0.297. Any specific sample taken differs, of course, from the donor's average value and this variability has been estimated to be 0.307 mg. per cent from the relation

\[ \sigma = \sqrt{\frac{\sum(X_i - \bar{x})^2 + \cdots + \sum(X_{17} - \bar{x})^2}{(n_1 - 1) + \cdots (n_{17} - 1)}} \]

If there was on the average no difference between donors, the standard deviation of the donor's average from 4.22 mg. per cent would be \( 0.307 / \sqrt{15.3} = 0.078 \) mg. per cent, where 15.3 is the average number of determinations per donor. Actually, as shown above, the value is 0.297, indicating that there is more difference between donors than would be expected from chance causes alone.

The question remains whether it is likely that any individual would cover the full range of possible amino nitrogen values, or whether a certain smaller range of values is preferred. In other words, statistically expressed, are all the observed values taken from the same source or from different sources?

Bartlett's \( \chi^2 \) test (7) has been used here.

\[ \text{Crude } \chi^2 = 2.303[(\log V_o) - (\Sigma df)(\log V)] \]

where \( V = \sigma^2 \), \( V_o = (\Sigma (df)(V))/\Sigma df \), \( df = (n - 1) \).

Both the crude and the corrected value of \( \chi^2 \) proved that the samples were not taken from the same source. A similar result was obtained through the analysis of the variance of the individual data and variance of the means.

It is physiologically significant that certain individuals have smaller variations from their mean values than other individuals who show wider variations. The causes of these individual differences in stability of plasma amino acid concentration remain to be investigated, but they may be related to the subject's emotional stability (4), since it has been shown that pharmacological stimulation of the sympathetic nervous system causes a marked drop in plasma amino nitrogen.

SUMMARY

1. Covering a period of about 3 months, an average of 15.3 samples of fasting blood has been taken from each of seventeen persons (three women and fourteen men) and the plasma amino nitrogen has been determined by the ninhydrin manometric method. Fig. 1 shows these data plotted against time.

2. The average amino nitrogen value of all the above 262 determinations was 4.26 mg. per cent.

3. Bartlett's \( \chi^2 \) test applied to the above figures and analysis of the vari-
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ances showed that it is very improbable that the above data were all drawn from the same source. It is, therefore, concluded that each individual develops his personal average amino nitrogen level and any given sample fluctuates around this individual average.

4. The true average amino nitrogen value is, therefore, more correctly determined by averaging the individual averages. This gave the average value of 4.22 mg. per cent amino nitrogen. In computing the confidence limits of this value, it was determined that there are 19 chances in 20 that the true average would lie within the limits of 4.07 and 4.37 mg. per cent.

5. The basal plasma amino nitrogen values of an individual do not seem to fluctuate greatly from day to day, but to develop upward or downward trends, which last for a limited number of days.

6. The range of amino nitrogen in the plasma is an individual function that remains fairly constant from month to month.

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